

CLAIMS

1. An enzyme exhibiting endo- β -1,4-glucanase activity (EC 3.2.1.4) which is selected from one of
- 5 (a) a polypeptide encoded by the DNA sequence of positions 76 to 1455 of SEQ ID NO:1;
- (b) a polypeptide produced by culturing a cell comprising the sequence of SEQ ID NO:1 under conditions wherein the DNA sequence is expressed;
- 10 (c) an endo- β -1,4-glucanase enzyme having a sequence of at least 75% identity to positions 26-485 of SEQ ID NO:2 polypeptide comprising an amino acid sequence derived from the amino acid sequence of positions 26-485 of SEQ ID NO:2 when identity is determined by GAP provided in
- 15 the GCG program package using a GAP creation penalty of 3.0 and GAP extension penalty of 0.1; and
- (d) a polypeptide encoded by the endoglucanase encoding part of the DNA sequence obtainable from the plasmid in *Escherichia coli* DSM 12805.
- 20 2. The enzyme according to claim 1, which belongs to family 9 of glycosyl hydrolases.
3. The enzyme according to claim 1 or 2, which comprises a
- 25 polypeptide endogeneous to *Bacillus licheniformis*, ATCC 14580.
4. The enzyme according to any of claims 1-3, which is active at a pH in the range of 4-11, preferably 5.5-10.5.
- 30 5. The enzyme according to claim 1 which is
- (a) a polypeptide comprising an amino acid sequence as shown in positions 26-646 of SEQ ID NO:2, or
- (b) an analogue of the polypeptide which is at least 75% homologous with the polypeptide.

6. An isolated polynucleotide molecule encoding a polypeptide having endo-beta-1,4-endoglucanase activity selected from the group consisting of:

- 5 (a) polynucleotide molecules comprising a nucleotide sequence as shown in SEQ ID NO:1 from nucleotide 76 to nucleotide 1455;
- (b) species homologs of (a);
- (c) polynucleotide molecules that encode a polypeptide that is at least 75% identical to the amino acid sequence of SEQ ID NO:2 from amino acid residue 26 to amino acid residue 485;
- 10 (d) molecules complementary to (a), (b), or (c); and
- (e) degenerate nucleotide sequences of (a) or (b).

7. The polynucleotide molecule according to claim 6 which is selected from the group consisting of:

- 15 (a) polynucleotide molecules comprising a nucleotide sequence as shown in SEQ ID NO:1 from nucleotide 76 to nucleotide 1941;
- (b) species homologs of (a);
- (c) polynucleotide molecules that encode a polypeptide that is at least 75% identical to the amino acid sequence of SEQ ID NO:2
- 20 from amino acid residue 26 to amino acid residue 646;
- (d) molecules complementary to (a), (b), or (c); and
- (e) degenerate nucleotide sequences of (a) or (b).

8. The isolated polynucleotide molecule according to claim 6 or
25 7, wherein the polynucleotide is DNA.

9. An isolated polynucleotide molecule encoding a polypeptide having endo-beta-1,4-glucanase activity which polynucleotide molecule hybridizes to a denatured double-stranded DNA probe under medium stringency conditions, wherein the probe is selected
30 from the group consisting of DNA probes comprising the sequence shown in positions 76-1455 of SEQ ID NO:1 and DNA probes comprising a subsequence of positions 76-1455 of SEQ ID NO:1 having a length of at least about 100 base pairs.

35 10. The isolated polynucleotide molecule according to claim 6 which is isolated from or produced on the basis of a DNA library

from a prokaryot, preferably from a bacterium, more preferably from a gram positive bacterium.

11. The isolated polynucleotide molecule according to claim 10
5 which is isolated from or produced on the basis of a DNA library from a strain belonging to the genus *Bacillus*, in particular a strain of *Bacillus licheniformis*, especially *Bacillus licheniformis*, ATCC 14580.
- 10 12. The isolated polynucleotide molecule according to any of the claims 6-11 which is isolated from *Escherichia coli*, DSM 12805.
13. An expression vector comprising the following operably
linked elements: a transcription promoter; a DNA segment se-
15 lected from the group consisting of (a) polynucleotide molecules encoding a polypeptide having endo-beta-1,4-glucanase activity comprising a nucleotide sequence as shown in SEQ ID NO:1 from nucleotide 76 to nucleotide 1455, (b) polynucleotide molecules encoding a polypeptide having endo-beta-1,4-glucanase activity
20 that is at least 75% identical to the amino acid sequence of SEQ ID NO:2 from amino acid residue 26 to amino acid residue 485, and (c) degenerate nucleotide sequences of (a) or (b); and a transcription terminator.
- 25 14. A cultured cell into which has been introduced an expression vector according to claim 13, wherein said cell expresses the polypeptide encoded by the DNA segment.
15. The cell according to claim 14, which is a prokaryotic cell,
30 in particular a bacterial cell, or an endogenous cell from which the DNA segment, encoding the polypeptide exhibiting endo-beta-1,4-glucanase activity, originates.
16. The cell according to claim 15, wherein the cell belongs to
35 a strain of *Bacillus*, preferably a strain of *Bacillus subtilis* or *Bacillus lentus*.

17. A cell according to claim 15, wherein the cell belongs to a strain of *Bacillus licheniformis*, preferably *Bacillus licheniformis*, ATCC 14580.

5 18. The cell according to claim 15, wherein the cell belongs to a strain of *Pseudomonas*, preferably a strain of *Pseudomonas fluorescens* or *Pseudomonas mendocina*.

10 19. The cell according to claim 14, wherein the cell belongs to a strain of *Streptomyces*.

20. A cell according to claim 14 wherein the cell belongs to a strain of *Saccharomyces*, preferably a strain of *Saccharomyces cerevisiae*.

15 21. A method of producing a polypeptide having endo-beta-1,4-glucanase activity comprising culturing a cell into which has been introduced an expression vector according to claim 13, whereby said cell expresses a polypeptide encoded by the DNA
20 segment; and recovering the polypeptide.

22. An enzyme composition comprising the enzyme according to claim 1.

25 23. The composition according to claim 22 which further comprises one or more enzymes selected from the group consisting of proteases, cellulases (endoglucanases), β -glucanases, hemicellulases, lipases, peroxidases, laccases, α -amylases, glucoamylases, cutinases, pectinases, reductases, oxidases, phenoloxi-
30 dases, ligninases, pullulanases, pectate lyases, xyloglucanases, xylanases, pectin acetyl esterases, polygalacturonases, rhamnogalacturonases, pectin lyases, other mannanases, pectin methyl esterases, cellobiohydrolases, transglutaminases; or mixtures thereof.

24. An isolated enzyme having endo-beta-1,4-glucanase activity, in which the enzyme is (i) free from homologous impurities, and (ii) produced by the method according to claim 21.
- 5 25. An isolated substantially pure biological culture of the strain *Escherichia coli*, DSM 12805.
- 10 26. A method for degradation of cellulose-containing biomass, wherein the biomass is treated with an effective amount of the enzyme according to any of claims 1-5 and 24 or of the enzyme composition according to claim 22 or 23.